UNIVERSITY OF PENNSYLVANIA

MICRO-INJECTION STUDIES OF CAPILLARY PERMEABILITY

EUGENE MARKLEY LANDIS

A THESIS

IN PHYSIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

PHILADELPHIA
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I. FACTORS IN THE PRODUCTION OF CAPILLARY STASIS

II. THE RELATION BETWEEN CAPILLARY PRESSURE AND THE RATE AT WHICH FLUID PASSES THROUGH THE WALLS OF SINGLE CAPILLARIES

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MICRO-INJECTION STUDIES OF CAPILLARY PERMEABILITY

I. Factors in the Production of Capillary Stasis

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From a study of the mechanism of lymph formation Starling (1896) concluded that since the capillary endothelium is normally impermeable to the proteins of the blood the movement of water through the capillary wall depends primarily upon the balance between capillary blood pressure and the osmotic pressure of the plasma colloids. An excess of capillary pressure would therefore cause water to pass toward the tissues, while a converse relation would lead to the movement of fluid into the blood. It is particularly emphasized that the vitality of the capillary wall is of paramount importance since injury increases its permeability to protein, with a corresponding reduction of the effective colloid osmotic pressure. According to this view, therefore, the direction and the amount of movement of water through the normal capillary wall is determined primarily by the level of capillary pressure in association with osmotic factors.

Krogh and Harrop (1921), however, observed that simultaneously with the capillary dilatation produced by urethane in 5 and in 25 per cent solutions the permeability of the endothelium increases. This is indicated microscopically by the development of "stasis," by which they refer to a concentration of the corpuscles produced by the rapid loss of plasma through a capillary wall whose permeability has been markedly increased. The viscosity of the concentrated blood causes flow first to become sluggish, and finally to cease entirely, as the venous capillaries become completely filled with solid cylinders of tightly packed corpuscles.

The term "stasis" as used by Elliott (1921), Lennartz (1921) and Stegemann (1924) merely designates a cessation of flow without reference either to escape of fluid, or to change in the ratio of corpuscles to plasma. In experiments dealing with capillary permeability, however, Krogh's use of the word has been adopted by Herzog (1925) and by Florey (1926) although in each case the important feature is the concentration of the red cells, to which stoppage of flow is incidental. To avoid confusion, however, it is in this latter sense that the word stasis is to be used in this paper.

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Krogh concludes (1922) that "no dilatation of capillaries involving mechanical stretching of the endothelium can take place without being accompanied by an increase in the permeability,—an increase which runs on the whole parallel to the degree of dilatation and which allows all the normal plasma colloids to filter off rapidly when the capillaries are strongly dilated." Comparison of the low pressures in the venous capillaries of the skin (Carrier and Rehberg, 1923) with the osmotic pressure of the plasma colloids, as determined in collodion sacs of graded permeability, led to the further conclusion that there is in almost all tissues an excess of the effective osmotic pressure over capillary blood pressure. Krogh therefore expresses the belief that capillary permeability depends primarily upon the degree of dilatation, while capillary pressure is of minor significance.

1. Factors to be considered in the production of stasis. The increase of permeability observed in urethane stasis is ascribed by Krogh and Harrop (1921) to pores having a diameter between five and two hundred millimicra which appear when the capillary wall is sufficiently stretched. Capillary pressure though not measured was believed to have no influence since stasis developed quite as easily after urethane, which leaves the arterioles relatively constricted, as after mechanical stimulation, in which case the marked arteriolar dilatation increases capillary pressure and flow. The possibility that urethane stasis might be related to the toxicity of the narcotic, or to the osmotic properties of the markedly hypertonic solutions used, is not considered.

There is evidence in the literature, however, that an increased passage of fluid through the capillary wall may be due to any one of several important factors of which the most obvious are: 1, increase of capillary pressure; 2, injury to the endothelium; 3, an osmotic withdrawal by hypertonic solutions, and 4, increased rate of blood flow with a rise of capillary pressure. In view of the evidence already accumulated in favor of Starling's view, it would seem that these more important possibilites should be directly eliminated before ascribing the increased permeability of any dilatation to an inherent change in the character of the capillary wall.

a. Capillary pressure. The low pressures found by Carrier and Rehberg (1923) in the venous capillaries of the skin tend to minimize the importance of capillary pressure as a factor in fluid interchange. However, the vessels penetrated were on the venous side of the capillary network. Kylin (1926) observes that with the use of pipettes having a diameter of 20 micra flow is blocked and the pressure actually measured is that of the vessel into which the penetrated venous capillary empties. Moreover, measurements made in but one portion of the peripheral circulation would not show the presence of a pressure gradient if one existed.

A series of direct measurements has been made by a micro-injection method at various points distributed through the peripheral circulation

of the frog's mesentery (Landis, 1926). Special precautions were taken to avoid any modification of flow in the observed capillary. By these determinations the presence of a gradient of pressure fall in the capillary bed has been established. In the arteriolar capillary the average pressure was 14.5 cm. water, and in the venous capillary 10.0 cm. White (1924) found the colloids of frog's plasma exerted an osmotic pressure ranging between 10 and 12 cm. of water. Hence the average capillary pressure in the arteriolar end is above, and in the venous end below, the colloid osmotic pressure. This indicates a balance between capillary pressure and the osmotic pressure of the plasma colloids as was originally suggested by Starling.

Moreover, direct measurements also indicated that probably in dilated capillaries and certainly in capillaries with high rates of flow there is a higher pressure than in constricted vessels. The independent variation of arterial and capillary pressures which is well established (Kylin, 1926) makes it impossible to infer the level of capillary pressure from measurements of systemic pressure, as is done by Tani (1924).

There is evidence also that a certain level of peripheral pressure is necessary for the development of edema. Tainter and Hanzlik (1924) found that following the intravenous administration of paraphenylenediamine, edema is prevented by lowering the peripheral pressure. This may be accomplished either by lowering systemic pressure with shock-producing doses of the drug, or by the administration of adrenalin, which though raising arterial pressure, lowers the capillary or filtration pressure. Hirschfelder (1924) found that the inflammatory edema of the conjunctiva following application of mustard oil could be prevented by peripheral vasoconstrictor agents such as cocaine, or adrenalin, which act by reducing capillary pressure. The importance of a sufficient filtration pressure is also indicated by the action of sodium nitrite. This vaso-dilator substance increases the inflammatory edema when locally applied; but when introduced by vein, though the same degree of dilatation is produced locally, no edema is observed because of the lowered systemic pressure. Hence the important element appears to be not dilatation but the increased pressure which accompanies it.

b. Injury to the endothelium. According to Starling any substance which by its toxicity partially or completely destroys the impermeability of the endothelium for plasma colloids would cause water to pass at an abnormally low capillary pressure. The gradual development of pulmonary edema in the heart-lung preparation is believed by Lambert and Gremels (1926) to be due to the injury of the pulmonary capillaries by toxic substances forming slowly in defibrinated blood. This damage could be histologically demonstrated. In the formation of urticarial wheals Lewis (1924) and Lewis and Grant (1924) have concluded that the

movement of fluid is independent of any increased permeability of vessel walls which might be due to distention, but is chiefly due to a heightened permeability caused by the liberation of a histamine-like substance after tissue injury.

The edema following paraphenylenediamine injection is believed by Tainter and Hanzlik (1924) to owe its gelatinous character to the abnormal permeability of the damaged endothelium for plasma proteins including fibrinogen. Hirschfelder (1924) also demonstrated by trypan blue that the inflammatory edema following application of mustard oil was due to a damaged endothelium and that epinephrin prevented the edema not by any direct protective action on the endothelium but by its reduction of peripheral pressure.

Jacobj (1923) describes the effects of applying formaldehyde to the frog web. There is observed first an increased flow, then a dilatation and concentration of the red cells with stasis. The description resembles that of Krogh for urethane stasis. The tendency of the corpusles to adhere to the capillary wall is given by Jacobj as evidence that both dilatation and increased permeability are the result of injury.

Herzog (1925) in repeating Krogh's experiments with 20 per cent urethane finds that in the frog's tongue capillaries thus dilated are not only more permeable but more sticky as well. Both effects are best observed in the areas rapidly passing into stasis, where, however, there is noted a definite tendency toward irreversibility. In addition the possibility of the toxic action of even dilute solutions of urethane is presented in the reduced or abolished reactivity of certain smooth muscles to pilocarpine and barium after their relaxation in 1 to 2 per cent urethane. Franklin (1925) found that a 1 per cent solution produces a relaxation from which recovery does not occur.

There is every indication that injury of the endothelium can increase permeability and there is some possibility as well that urethane itself is a toxic substance even in relatively small percentage.

c. Osmotic activity of the substance applied. Weed and McKibben (1919) described a diminution in brain size on the introduction of hypertonic salt solution into the blood stream. The results are explained as fundamentally osmotic effects, by which a hypertonic solution introduced into the blood stream withdraws water from the cranial cavity. If the hypertonic solution is applied outside the vascular system there is similarly a movement of water toward the higher osmotic pressure. Cushing and Foley (1920) found that concentrated salt solutions in the alimentary canal removed water from the intestinal capillaries, and then secondarily from the cranial cavity.

The isotonic strength of urethane is about 2.5 per cent. It has been noted by Krogh (1922) that a 1 per cent solution applied to the tongue

causes only increased rate of flow, and that 5 and 25 per cent both cause stasis. Since the latter solutions are markedly hypertonic and are the only ones effective in causing loss of fluid through the capillary wall, the possibility of an osmotic withdrawal should be ruled out before the concentration of the blood is ascribed to dilatation only.

d. Rate of blood flow in the observed capillary. In general, capillary pressure rises by amounts easily measurable by the direct micro-injection method as the rate of blood flow in the capillary increases. In abnormally permeable vessels, moreover, if active flow be maintained a minor loss of fluid is imperceptible. It becomes increasingly noticeable if the flow is slower and the same volume of blood is exposed to the concentrating factors for a longer period. This occurs as flow is made more sluggish in urethane stasis.

Lewis (1924) indicates that the formation of urticarial wheals is largely dependent upon the rapidity of blood flow in the stimulated area and apparently not related to mere distention.

It is the purpose of the experiments to be described to consider each of these factors in turn especially with relation to urethane stasis, upon which a certain amount of emphasis is placed in Krogh's hypothesis. The advantages of the micro-injection method lie in the possibility of observing and manipulating the single capillary while at the same time the pressure in the vessel is measured directly. The results indicate that certain of these factors play a great part in the production of stasis by urethane thus reducing the probable importance of dilatation and stretching as the sole cause of increased permeability.

2. Material and methods. The observations have been made upon the mesenteric capillaries of two species of frog (Rana pipiens and R. catesbiana). They are well adapted for observation, measurement of pressure, and the application of substances to their exterior. The use of a thin membrane lessens the possibility of dilution as penetration occurs.

The general method has already been described (Landis, 1926). The capillaries were viewed through a 16 mm. objective and a Zeiss photomicrographic ocular. Capillary pressure was measured through micropipettes having a diameter of 4–8 micra at the tip. The other end was connected to a column of water for the purpose of balancing the capillary pressure. A few minor improvements added to the ease and accuracy of making the determinations. For the sake of precision and firmness the frog board has been mounted on a mechanical stage. One of the major difficulties in the technique has been the ease with which the tips of the micro-pipettes are clogged by fine particles which are found, even after filtration, in the fluids to be injected. It has been advantageous to put the solutions for micro-injection into glass tubes of 2 mm. bore. After both ends are sealed in a flame the tubes are centrifuged at high speed for 3–5

minutes. The lower portion of each vessel with some of its contained fluid is broken off. This removes the detritus which clogs the micropipette and leaves the remaining solution clearer than any ordinary filtrate.

3. Observations. *a. Relation of high capillary pressure to passage of fluid.* Capillary pressures as high as 24 cm. of water have been observed in the normal mesentery. But the exceedingly rapid flow under these circumstances prevents the production of any observable concentration of the corpuscles. The passage of fluid at these high. virtually arteriolar, levels can be made obvious, however, by raising pressure and simultaneously stopping flow. This can be done by very gentle pressure of the microscopic glass rod upon the venous end of a capillary arising directly from a large arteriole in which flow is rapid.

This raises the pressure in the entire length of capillary to the arteriolar level. Under these conditions without any change in capillary diameter the corpuscles slowly move together as fluid is filtered through the wall. Even full arteriolar pressure, however, does not produce stasis if the capillary wall is normal, some plasma remaining between the corpuscles even after 15 minutes of pressure well above 25 cm. of water. The closely packed cylinder of corpuscles characteristic of stasis is not formed, presumably because with concentration the osmotic pressure of the colloids is increased until the higher filtration pressure is balanced. Immediately on the removal of the obstruction to flow the cells are washed out of the capillary into the venule and normal circulation is resumed.

The passage of fluid here cannot be due to lack of oxygen since the exposed mesentery is well aerated, nor to dilatation since the diameter remained constant. The concentration depends then upon a high filtration pressure. It can be stopped at any stage simply by the compression of the arteriole or of the arteriolar end of the capillary and is immediately resumed when the obstruction is removed from the arteriole and pressure again rises in the impeded capillary. When the arteriolar pressure is low, i.e., below 18 cm. water, there is little or no visible concentration. Even with the high pressures of 28–30 cm. water some plasma is retained due to the osmotic properties of the colloids to which the capillary wall, being normal, is impermeable.

b. The production of stasis by injury of the capillary wall. In certain preliminary experiments dye solutions were injected through a micropipette inserted into the capillary lumen. It was observed that the dye at once passed through the portion of capillary wall that had been damaged in the course of the insertion of the tip of the pipette. It required some minutes on the other hand for the dye to appear outside the normal endothelium. It was concluded, after ruling out the possibility of leakage that mechanical pressure increased the permeability of the capillary wall by an injury effect.

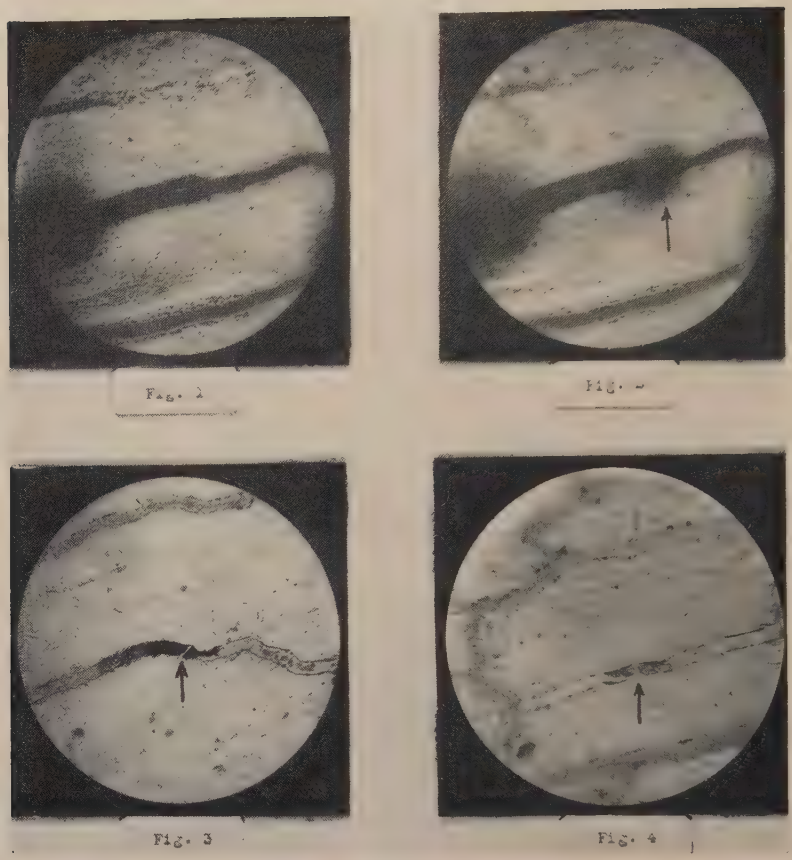

Fig. 1. Photomicrograph (× 80) showing the perfusion of a normal capillary with toluidine blue in Ringer's solution. After 4 minutes' flow at 10 cm. pressure there is no visible passage except to the left where the wall has been injured by the insertion of the micropipette.

Fig. 2. The same capillary is shown thirty seconds after the wall was locally compressed with the blunt glass rod. The point of injury is indicated by the arrow. The passage of dye in this area indicates the increased permeability resulting from injury.

Fig. 3. Photomicrograph (× 80) showing the increased permeability following mechanical injury. In contrast to the dye India ink is retained by the capillary wall as the plasma escapes, indicating that the increased permeability is not due to a tear of the endothelium. Concentration of the ink, and hence filtration of plasma, was greatest on the arteriolar side, to the left. Arrow denotes the injured area.

Fig. 4. Photomicrograph (× 80) showing local concentration of cells and stasis produced by mechanical injury. Arteriole is to the left. Arrow indicates the injured area.

This increase of permeability to a Ringer's solution containing dissolved dye is indicated in figures 1 and 2. The micro-pipette was introduced at the left of the upper capillary and dye perfused, the pressure being 10 cm. water. At the end of four minutes the first photomicrograph was taken (fig. 1) showing no perceptible passage at any point except on the left where the pipette has injured the wall. At this time the capillary was compressed by the glass rod and a second photograph taken 30 seconds later shows a marked passage of the dye solution through the injured area, while there is still no passage through the normal capillary wall.

The fluid does not spurt from a single point as would be the case if the endothelium had been ruptured but filters outward uniformly along the whole damaged section. To demonstrate that compression does not produce a tear in the capillary wall, India ink was introduced into the ventricle of a frog until the plasma became a homogeneous grey in color. If a capillary is then injured by compression as before, even after severe pressure the India ink does not appear outside the wall, but is concentrated along with the corpuscles as plasma escapes (fig. 3). In fact the India ink outlines the damaged area very clearly by adhering to the abnormally permeable portion of the endothelium. Finally corpuscles and black pigment are commingled in the plasma-free collection characteristic of stasis.

These results indicate that in mechanical injury there is a marked increase in permeability which, while permitting the immediate passage of dye in Ringer's solution, is not sufficient to allow the particles of India ink to pass. This is therefore an increased permeability without change in capillary diameter. Flow has been stopped by the introduction of the micro-pipette and pressure throughout the length of the capillary is that of the arteriole or venule to which it is connected. Differences in filtration pressure cannot explain the results since two areas of a single capillary are being compared. In this instance then injury of the endothelium seems to be the sole factor in the change of permeability.

According to Krogh (1922) India ink is similarly filtered off in the concentration and stasis produced by urethane. Herzog (1925) describes the aggregation of India ink particles on the inner surface of the endothelium wherever stasis is produced whether by mechanical pinching, application of urethane, injection of arsenical compounds or by hot water.

Typical concentration of the red cells and stasis can also be produced by exerting firm pressure on a normal capillary by means of the blunt rod. With the continued loss of plasma through the injured area the corpuscles move slowly toward the damaged zone from both extremities of the capillary. The slowness of movement leads to plasma skimming at the two extremities of the vessel since movement of fluid is not rapid enough to cause corpuscles to leave the axial stream of either arteriole or venule. The mechanism of this plasma skimming has been described previously (Landis,

1926). Finally there results the condition photographed in figure 4. The cells which previously were equally distributed throughout the entire length of the capillary are now packed in one mass, the remainder of the vessel being filled with plasma alone.

When a large venous capillary is thus compressed concentration and stasis occur in the same manner. But usually the central cells in the mass are slowly pushed forward by the pressure on the arterial side. Finally they are forced completely through and circulation is resumed through a hollow cylinder of erythrocytes which are closely adherent to the damaged endothelium. This stickiness of the injured wall is very characteristically seen after the capillary is injured either chemically or mechanically.

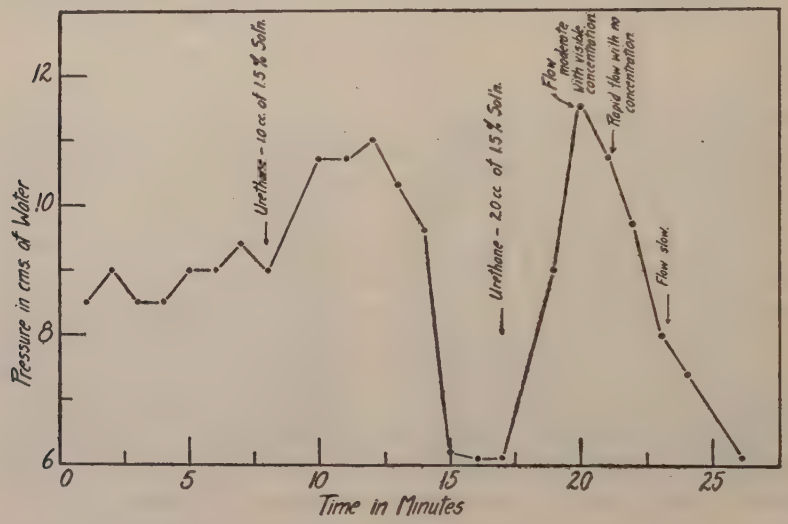

Fig. 5. Chart showing rise in capillary pressure following two applications of 1.5 per cent urethane in Ringer's solution to the mesentery.

Stasis of this sort while independent of change in capillary diameter is to some extent dependent on capillary pressure. The rapidity with which concentration is effected is modified by the filtration pressure. The movement of the corpuscles toward the abnormally permeable area is rhythmic in character, the chief passage of fluid occurring during systole when pressure is high. The collection of cells or of India ink occurs much more rapidly on the arteriolar side than on the venous side (fig. 3) and indeed concentration may be entirely absent on the side of lower capillary pressure. In capillary nets with higher rates of flow, accompanied by increased capillary pressure, concentration and stasis occur more rapidly. If the pressure is generally low even severe injury is often unproductive of

stasis. Moreover the concentration may be immediately stopped at any stage if the arteriole is blocked and pressure thereby allowed to fall.

It seems clear that in mechanical injury there is an increased permeability of the capillary wall, and the stasis thus produced is independent of diameter change, but is modified by the level of capillary pressure. In addition, stasis has been produced by a series of substances which act by killing the tissues. Water of sufficiently high temperature, acids and alkalies, formaldehyde, ether, chloroform and alcohol in sufficient strength all increase the permeability to this extreme extent. In each case the first notable change is the tendency of the erythrocytes to stick fast to the

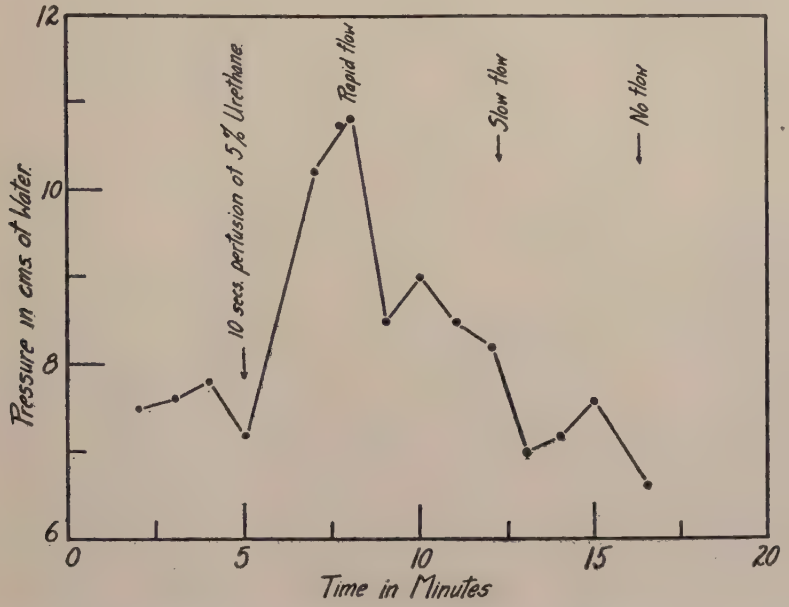

Fig. 6. Chart showing rise in capillary pressure following injection of 1.5 per cent urethane in Ringer's solution into a capillary network for 10 seconds.

endothelium, which is very shortly followed by the development of typical stasis.

c. The stasis produced by urethane. *(1) The effect of urethane on capillary pressure.* Direct measurement indicates that urethane in 1 per cent strength, which produces only a moderate dilatation and increased flow without stasis, increases capillary pressure by 2 to 6 cm. of water simultaneously with the increase in flow. Figure 5 shows the rise of capillary pressure obtained by the application of 1.5 per cent urethane in Ringer's solution, which sometimes produces a slight concentration when the pressure rises above 11 cm. If the urethane be introduced by micro-pipette into a

capillary network the same effects are noted. Figure 6 indicates a rise of capillary pressure of almost 4 cm. after ten seconds' local perfusion back to the arteriole with 1.5 per cent urethane. Direct measurement therefore shows rise in pressure coincidently with the slight initial dilatation and increased flow, which would tend to cause a greater passage of fluid from the blood stream.

When higher percentages of urethane are used, simultaneously with the sluggish flow and the beginning of concentration there is a marked and sudden rise of pressure to the level characteristic of arteriolar pressure, i.e., in excess of 25 cm. This marked rise of pressure (fig. 7) is due to the

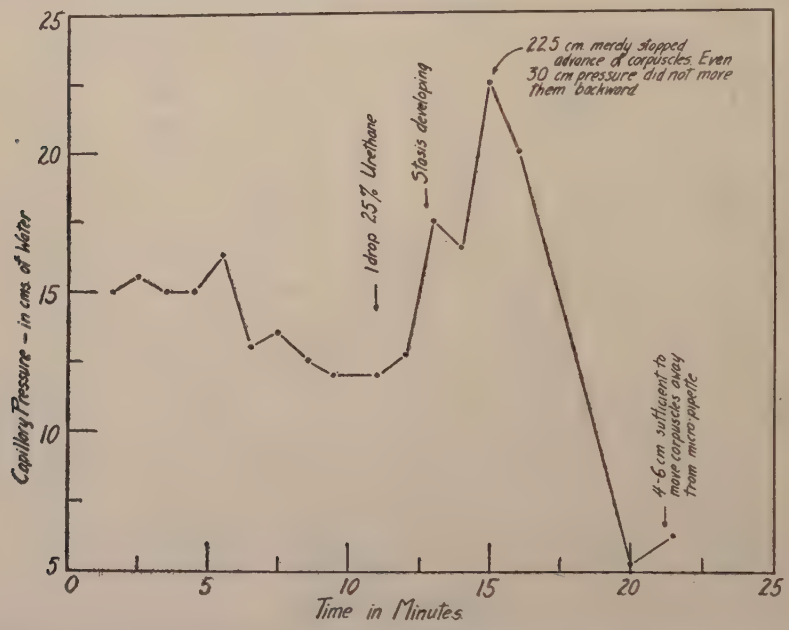

Fig. 7. Chart indicating the changes in capillary pressure occurring during the production of stasis by 25 per cent urethane.

blockage of the venous capillaries. If, as the pressure is rising, the cells collected in the venous capillary are washed out by a strong current of Ringer's solution from the pipette the pressure falls, only to rise again as the leakage of plasma once more makes the blood more viscous and impedes venous outflow.

With the reduction and finally the total stoppage of venous outflow, capillary pressure in the affected region rises until it equals arteriolar pressure. Plasma-skimming then results and this somewhat reduces the number of cells entering the vessels passing into stasis. Nevertheless due to the higher pressure the passage of fluid is quite rapid, and those

cells entering the capillary are packed together forming a solid cylinder which gradually extends from the venous to the arteriolar extremity of the capillary network. As this occurs the pressure is high manifestly due to a blocked venous outflow, producing the peak in capillary pressure shown in figure 7 during early stasis.

A few minutes later, however, 4 to 6 cm. water pressure is commonly sufficient to cause the corpuscles to move away from the tip of the pipette. This striking fall of capillary pressure occurs after the progressive packing of cells has reached the ends of the finest arterioles. It occurs because the cells collected in the ends of the arterioles have interposed a block to the arterial pressure.

That a degree of capillary pressure apparently something above 10 cm. is required to produce stasis with urethane is indicated by the cessation of concentration when arteriolar flow is blocked. Stasis is difficult to obtain when the animal is partially exsanguinated, probably due to the very low peripheral pressures resulting from arteriolar constriction. In addition in the normal animal the movement of corpuscles here, as in mechanical injury, is pulsatile in character, more rapid during systole than during diastole, indicating that capillary pressure is a factor in determining the rate of filtration of plasma.

The increase of pressure, however, cannot be the chief factor in the production of urethane stasis, since it has already been shown that in a normal capillary a pressure equivalent to that in the arteriole is insufficient to cause stasis, even when flow is stopped. The initial rise of 2 to 6 cm. therefore while aiding passage of fluid cannot be the real cause of stasis. The excessive rise in pressure later is the result first of increased viscosity of the concentrated blood, and later of absolute block of venous outflow. This high pressure while aiding the concentration is not the cause of stasis but one of its effects.

(*2*) *Relation of stasis to dilatation.* If Krogh's hypothesis is correct stasis should only occur in vessels which dilate at the time the increased permeability becomes evident. In the frog mesentery as prepared for these experiments the capillaries are as a rule moderately dilated within a few minutes after the tissues are exposed. Nevertheless circulation proceeds normally for hours if the surface is kept carefully covered with Ringer's solution. On the application of urethane to these dilated capillaries, stasis has frequently been observed to occur without measurable increase in the diameter of the vessels affected.

Photomicrographs have been prepared showing a capillary network before application of urethane and afterward when in stasis. A typical comparison of the diameters of the same capillaries before and after stasis is given in table 1.

With the higher concentrations even constricted capillaries frequently

pass rapidly into stasis, without change in diameter. Thus the corpuscles were barely able to enter the 4 constricted capillaries shown in stasis in figure 8.

Stasis produced by concentrated urethane is usually irreversible but with 1.5 per cent urethane in Ringer's solution flow is sometimes resumed. An example of such reversible stasis is represented in figures 9 to 11. The normal flow preceding the experiment is shown in figure 9. The short capillary *a* was only partially occupied by moving corpuscles. Due to this partial plasma-skimming one endothelial wall is clearly visible. With the sluggish flow of early stasis the stream of corpuscles was widened until it occupied the entire lumen. The appearance was that of dilatation but the difference lay only in the degree to which the lumen was filled with cells. By actual measurement the diameter of this capillary remained the same, though 13 minutes later the vessel passed into complete stasis. The distance between the endothelial walls had not changed during this period of increased permeability. Figure 10 shows the vessel in stasis.

TABLE 1

CAPILLARY NUMBER	CAPILLARY DIAMETER BEFORE URETHANE AND WITH NORMAL CIRCULATION	CAPILLARY DIAMETER AFTER URETHANE AND WITH COMPLETE STASIS
	micra	*micra*
1	23	23
2	9	10
3	17	16
4	16	16
5	17	20

Furthermore after a short period this stasis resolved with resumption of flow, but there was no measurable change in capillary diameter as shown in figure 11.

Though circulation returned after a short period of stasis it is to be noted that a few corpuscles have adhered to the endothelium. This recalls the stickiness of the mechanically or chemically injured capillary wall and suggests the possibility that the increased permeability produced by urethane may be due to toxicity.

(*3*) *The toxicity of urethane.* The stasis of urethane is almost always irreversible. In addition strong solutions (20 to 25 per cent) cause the corpuscles within the capillaries and the melanophores in the mesentery to disintegrate and give up their pigment. Stickiness of the endothelium appears to a marked degree when 5 per cent urethane is injected into the lumen of a capillary, and as mentioned above even in a reversible stasis produced by 1.5 per cent urethane. All these points suggest an injury of the capillary wall by the narcotic.

To determine the degree of toxicity for living cells, Arbacia eggs were fertilized and placed for development into sea water to which had been added urethane. A 2 per cent solution of urethane in sea water killed

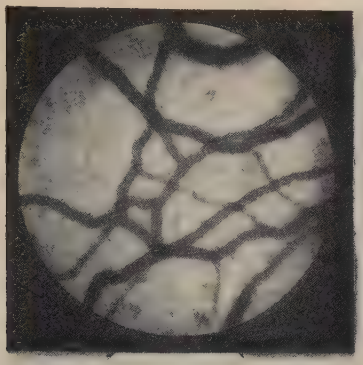

Fig. 8

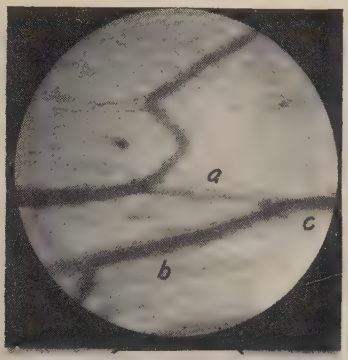

Fig. 9

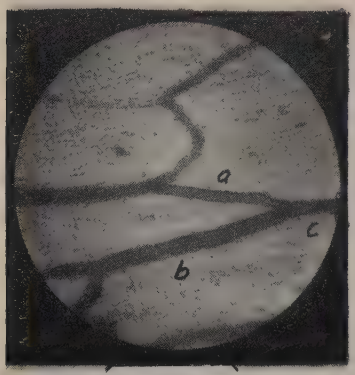

Fig. 10

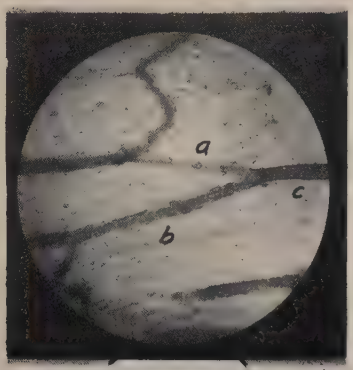

Fig. 11

Fig. 8. Photomicrograph (× 80) to show concentration of corpuscles and stasis in constricted capillaries after urethane.

Fig. 9. Photomicrograph (× 80) showing circulation before the application of 1.5 per cent urethane in Ringer's solution to the mesentery.

Fig. 10. Photomicrograph of same field taken 13 minutes later showing total stasis in *a* without measurable change in diameter. The venous capillaries *b* and *c* are also in stasis, but in the remaining vessels circulation still persists.

Fig. 11. Same field about 20 minutes later showing resumption of flow in *a* and *b* after urethane stasis without change in diameter. The corpuscles adherent to the walls of the capillary and venous capillary suggest injury of the endothelium.

all the eggs in the one-celled stage. Half of those in 1 per cent urethane failed to divide and none divided more than twice. In 0.5 per cent development was delayed but otherwise appeared fairly normal.

In addition the Arbacia eggs were exposed for increasing periods to solutions of urethane in sea water. After two rinsings they were returned to pure sea water and their development followed. An exposure of one minute to 25 per cent urethane in sea water sufficed to kill the eggs with a liberation of their pigment, much as the corpuscles give up hemoglobin in the presence of this strength. All the eggs were killed after a 5-minute exposure to 5 per cent urethane; 90 per cent were killed after two minutes, while with a one minute exposure the cleavages were delayed and markedly abnormal. With 1.5 per cent solution the early divisions were slightly delayed by the five minute exposure, while there was no perceptible effect produced by 1 minute in the solution.

Similar results were secured by observing motility of Paramecium caudatum in hay infusion to which urethane had been added. In 25 per cent and 15 per cent solutions death and cytoplasmic disintegration was immediate. Five per cent urethane immediately slowed their movement and killed within 5 to 7 minutes; while 1.5 per cent though markedly slowing movement after 5 minutes, did not kill in 20 minutes.

There is evidence therefore that urethane in the stasis-producing concentrations is a toxic substance and the increased permeability produced by this narcotic is believed to be due therefore primarily to injury of the capillary wall, and not to dilatation. Where dilatation does occur it is secondary to the tissue damage.

(*4*) *The osmotic properties of urethane in the production of stasis.* The ineffectiveness of an isotonic solution and the marked concentration with the hypertonic 5 per cent and 25 per cent solutions suggest an osmotic withdrawal of fluid. It has been found impossible, however, to demonstrate any such action, apparently because of the rapid penetration of the capillary wall by the drug. There is not sufficient semi-permeability to set up more than a transient osmotic flow of water toward the hypertonic solution.

A 2.5 per cent solution of urethane in distilled water (the isotonic strength) produces the same effects upon corpuscles and the capillary wall as distilled water alone. If urethane produces concentration by osmotic activity it should be devoid of effect if introduced by micro-pipette into the capillary lumen. It was found, on the contrary, that injection of 1.5 per cent as well as the higher concentrations was accompanied by the production of stasis in the vessels through which the solution passed. The effect must be a direct injury, therefore, since osmotic withdrawal cannot act in such a case.

Moreover, all attempts to show directly the osmotic withdrawal of fluid have been unsuccessful. A capillary was closed by gentle compression at its two extremities, thus forming a sac of endothelium filled with plasma. The application of 5 per cent urethane did not produce a measurable

change in diameter and the cells were not noticeably concentrated. As soon, however, as the obstructions were removed the pressure caused rapid production of stasis. It is concluded therefore that osmotic factors play little part, that the permeability increase is due chiefly to direct injury.

Discussion. Krogh's theory of capillary permeability rests partly upon the assumption that the increased passage of water observed after the application of urethane to the peripheral circulation is due to the capillary dilatation produced. Consideration of the other possible factors has indicated that this is not the case. The two primary factors in urethane stasis appear to be injury of the endothelium accompanied by a high capillary pressure.

It is shown that abnormally high filtration pressures alone, though producing visible concentration do not produce stasis, probably since the normal wall is impermeable to the plasma colloids. The primary cause of the increased permeability after urethane is the toxic effect of the drug on the capillary wall. The rise of pressure is the result of the blockage of venous outflow, and while aiding the production of stasis it does not initiate the concentration of corpuscles. Lambert and Gremels (1926) similarly found that a rise of pulmonary arterial pressure accompanies the production of pulmonary edema in the heart-lung preparation. They conclude that the rise in pressure is the result of the increased viscosity of the concentrated blood and that injury is therefore the primary cause of the abnormal passage of fluid.

These experiments further indicate the importance of the determination of capillary pressures in experiments having to do with permeability of the capillary wall. It is impossible by any method to infer from arterial pressure determinations what may be the level of capillary pressure at any moment because of the complexity of the factors concerned in peripheral resistance. Frequently pressure is not measured, or it is thought sufficient to record arterial pressures. That the latter may be actually misleading rather than helpful is indicated in the effect of adrenalin which in preventing edema produces all the effects of a lowered capillary pressure, and yet increases the arterial pressure.

An increased passage of fluid has been observed in various forms of active hyperemia. Rogowicz (1885) found that stimulation of the lingual nerve increased the rate of passage of dye through the walls of the tongue capillaries. Cutting the sympathetic resulted in a more rapid staining of the ear on the affected side. Local heating of the skin, which produces peripheral dilatation and rapid flow was observed by Okuneff (1924) to increase the passage of trypan blue from the blood stream into the heated tissues. Anitschkov (1924) in reviewing the mechanism of the passage of colloidal dyes through the endothelium concludes that any active hyperemia increases the passage of dye.

The study of urethane stasis has indicated that there is no close correspondence between capillary diameter and the degree of permeability. Direct measurements by micro-injection have indicated a balance between capillary pressure and the osmotic pressure of the plasma colloids in the frog. It seems probable therefore that the explanation of the increased filtration of active hyperemia rests with the rise in capillary pressure which accompanies arteriolar and capillary dilatation, rather than the increased permeability supposed to exist in the stretched endothelium of the dilated capillary.

The consideration of the various possible factors in fluid movement will probably reduce the number of substances which are now believed to act by a direct modification of the normal endothelial permeability. Petersen, Levinson and Hughes (1923) found that epinephrin causes chiefly a diminution in the quantity and protein content of the lymph, and attribute its action to an "inhibition" of the endothelium. Injection of pituitrin, which resembles epinephrin in its effect on peripheral tonus has been shown by Bayley, Davis, Whitman and Scott (1925) to diminish the volume of lymph collected from the thoracic duct and to delay the exit of physiological salt solution from the blood stream. They attribute the result to direct action of pituitrin on the endothelium. Lee (1925) has found that histamine produces a marked fall in cerebrospinal pressure coincidentally with the lowering of arterial and venous pressure. Microscopic observation through a window in the skull provided evidence of dehydration of the brain substance. Though recognizing that the effects are complicated by circulatory factors, he postulates the functional difference of the cerebral capillaries and advocates the consideration of an active participation of the endothelial cells in explaining the phenomena of capillary permeability. Capillary pressure determinations in instances of this kind would be of the greatest interest.

Definite proof of the quantitative action of capillary pressure as distinct from systemic blood pressure on the passage of fluid through the endothelium is lacking. But there is sufficient indication of its importance as a factor in both normal and injured vessels to require that it be measured in any problem concerned with capillary permeability. The accumulation of such data will be of the greatest value in determining the true mechanism of the transfer of water and dissolved substances through the capillary wall.

SUMMARY

The possible factors in the production of concentration of corpuscles within the capillary are considered.

In the capillaries of frog mesentery a rise of capillary pressure to the

arteriolar level is accompanied by a slow concentration of corpuscles, but the filtration of fluid is not sufficient to cause stasis.

Typical concentration of cells and stasis, such as described for dilated capillaries by Krogh, can be produced locally by mechanical injury without change in diameter. The filtration of plasma is influenced by capillary pressure.

Direct measurement by a micro-injection method indicates that dilute urethane causes a rise in capillary pressure of 2 to 6 cm. of water, later followed by a further increase to above 25 cm. as stasis appears in the venous capillaries.

Increased permeability to urethane has been observed to occur in constricted and in dilated capillaries without change in diameter. In the strengths required to cause increase of endothelial permeability, urethane kills Paramecium caudatum and developing marine eggs.

In view of these findings it appears that the increased passage of fluid observed after application of urethane is not due to dilatation but to injury of the capillary wall, accompanied by an increase in capillary pressure.

These facts are opposed to Krogh's theory of capillary permeability, which is based largely on the view that the increased permeability after the application of urethane is due solely to dilatation.

It is a pleasure to express my gratitude to Prof. M. H. Jacobs who first suggested the application of the micro-injection technique to the problems of capillary permeability.

BIBLIOGRAPHY

Anitschkov, N. 1924. Klin. Wochenschr., iii, 1729.
Bayley, Davis, Whitman and Scott. 1925. Proc. Soc. Exper. Biol. and Med., xxii, 312.
Carrier and Rehberg. 1923. Skand. Arch. f. Physiol., xliv, 20.
Cushing and Foley. 1920. Proc. Soc. Exper. Biol. and Med., xvii, 217.
Elliott, A. R. 1921. Journ. Amer. Med. Assoc., lxxvi, 1467.
Florey, H. 1926. Proc. Royal Soc. Ser. B., c, 269.
Franklin, K. J. 1925. Journ. Pharm. Exper. Therap., xxvi, 227.
Herzog, F. 1925. Virchow's Arch., cclvi, 1.
Hirschfelder, A. D. 1924. This Journal, lxx, 507.
Jacobj, W. 1923. Arch. Exper. Path. u. Pharm., xcviii, 55.
Krogh, A. 1922. Anatomy and physiology of capillaries. Yale Univ. Press.
Krogh and Harrop. 1921. Proc. Physiol. Soc., Journ. Physiol., liv, cxxv.
Kylin, E. 1926. Die Hypertöniekrankheiten. Springer, Berlin.
Lambert and Gremels. 1926. Journ. Physiol., lxi, 98.
Landis, E. M. 1926. This Journal, lxxv, 548.
Lee, F. C. 1925. This Journal, lxxiv, 317, 326.
Lennartz, E. 1921. Pflüger's Arch., cxci, 302.
Lewis, T. 1924. Heart, xi, 119.
Lewis and Grant. 1924. Heart, xi, 209.

Okuneff, N. 1924. Pflüger's Arch., cciv, 261.
Petersen, Levinson and Hughes. 1923. Journ. Immunol., viii, 323.
Rogowicz. 1885. Pflüger's Arch., xxxvi, 252.
Starling, E. H. 1896. Journ. Physiol., xix, 312.
Stegemann, H. 1924. Klin. Wochenschr., iii, 1163.
Tainter and Hanzlik. 1924. Journ. Pharm. Exper. Therap., xxiv, 179.
Tani, I. 1924. Biochem. Zeitschr., cxlv, 189.
Weed and McKibben. 1919. This Journal, xlviii, 531.
White, H. L. 1924. This Journal, lxviii, 523.

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MICRO-INJECTION STUDIES OF CAPILLARY PERMEABILITY

II. The Relation Between Capillary Pressure and the Rate at Which Fluid Passes Through the Walls of Single Capillaries

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Previous work on the vessels of the frog mesentery by the micro-injection method (Landis, 1926a) has indicated a balance between the average capillary blood pressure and the osmotic pressure of the plasma proteins. The fact that the average pressure in the arteriolar capillary is above—and in the venous capillary is below—the osmotic pressure of the plasma colloids is regarded as evidence favoring the hypothesis of Starling (1896). According to this view fluid leaves the blood stream when capillary pressure rises above the osmotic pressure of the plasma proteins, to which the normal endothelium is impermeable; and enters the blood stream when the capillary pressure falls below that level.

This paper deals with further experiments designed to study the permeability of the capillary wall in a quantitative manner, with control of capillary pressure. The experiments fall into two groups. In the first the movement of fluid through the capillary wall is identified indirectly by injecting solutions of certain easily visible dyes; in the second the rate of fluid passage inward or outward through the endothelium is measured directly by the respective dilution or concentration of the corpuscles in the capillary. Both methods provide further evidence of a more direct character favoring the view that fluid movement depends upon the level of capillary pressure in relation to the effective osmotic pressure of the plasma colloids.

I. The passage of dye solutions in relation to capillary pressure and dilatation. The rate of movement of dye solutions through the

capillary wall has been believed to depend on various chemical and physical qualities of the dyes used (Wittgenstein and Krebs, 1926; Schuleman, 1917; Schimidzu, 1922; Pohle, 1924). With any single dye solution, however, the state of the capillary wall itself has usually been regarded as the chief factor in determining the rate of passage. No account of the possible effect of changes in capillary pressure has been found in the literature.

Rogowicz (1885) observed that a dye introduced into the saphenous vein passed into the tissues more rapidly whenever active hyperemia was induced by stimulation of the vaso-dilator nerves or by division of the vaso-constrictor fibers. Krogh (1922) states that brilliant vital red and Chicago blue 6B, both relatively indiffusible substances, pass very slowly through the normal capillary wall; with the production of hyperemia or stasis, however, the vessels are quickly surrounded by a deeply stained zone. Capillary pressure, though not measured, was believed by Krogh to be a factor of slight importance. It was concluded that owing to a mechanical stretching of the endothelium the dilated capillary wall becomes more permeable even to colloidal substances. The active hyperemia resulting from local heating of the skin (Okuneff, 1924) produces in the affected area a more marked staining of the tissues after intravenous injection of trypan blue. Anitschkov (1924) also attributed this effect to local dilatation of the capillaries.

The micro-injection studies before cited (Landis, 1926b) have shown, however, that with an increase in the rate of flow, such as accompanies the dilatation and active hyperemia produced by heat or very dilute urethane, there is a marked rise in capillary pressure. It seems possible therefore that the increased passage of dye under these conditions may be related to this rise of capillary pressure rather than to the "stretching of the capillary wall" in dilatation. The experiments to be described were undertaken with the view of determining whether the passage of certain dyes is related to the level of capillary pressure, as would be expected from the Starling hypothesis, or to capillary diameter as suggested by Krogh.

Material and methods used in the dye experiments. The capillaries in the mesentery of the frog (Rana pipiens and R. catesbiana) were used throughout. The animals were pithed in the brain, the spinal cord remaining intact. The mesentery was delivered through a lateral incision, which had been cauterized lightly to prevent blood loss. After exposure the preparation was kept moist by a stream of Ringer's solution of pH 8.2 to 8.4 from a reservoir mounted on the frog board. The capillaries were observed through a 16 mm. objective and a Zeiss photomicrographic ocular.

Single capillaries were perfused at known pressures by means of the micro-injection apparatus previously described in detail. Flow in the vessel to be perfused was temporarily stopped at *a* (fig. 1) by pressure with the microscopic glass rod, to prevent the blood plasma from mixing with the

injected solutions. The micro-pipette, filled with the dye solution and attached to the apparatus for measuring pressure, was inserted at *b*. A slow flow of dye through the vessel was produced (fig. 2) by raising the pressure on the solution in the micro-pipette 1 to 2 cm. above the observed capillary pressure. In this way the corpuscles and plasma were removed and the fluid in the observed capillary consisted only of a constantly renewed Ringer's solution containing a known amount of dye.

Preliminary experiments (1927a) indicated that vessels of like diameter, or those arising from the same arteriole, frequently appear to present very different grades of permeability to dye solutions. It was found that these variations depend upon the level of capillary pressure, which is different not only in adjacent capillaries but changes in the same vessel from moment to moment. In each injection it was necessary therefore to measure pressure in the observed capillary at least before and after, and frequently also during, the period of perfusion.

As soon as the dye appeared in barely visible amount (fig. 2) outside the capillary wall the perfusion was stopped and the time recorded. Capillary diameter was measured with an ocular micrometer, during or immediately after the micro-injection. The passage of dye was observed at a distance of 200 to 500 micra from the point where the micro-pipette pierced the capillary in order that the results might not be complicated by injury of the wall. Finally in many instances the circulation was permitted to return to the vessel in order to observe whether the wall had been injured by the perfusion, as indicated by the abnormal loss of plasma and the production of stasis.

It was necessary to exercise extreme precaution against injury to the blood vessels in manipulating the mesentery during preparation. It was found that poor circulation, due either to hemorrhage or to pithing of the cord, leads to erratic results, since dyes pass with extreme rapidity, even at low pressure, through the capillary walls of a dead or moribund animal.

The filtration of toluidine blue in relation to capillary pressure and capillary diameter. Toluidine blue[1] is well adapted for micro-injection of this type because of its intensity of color and low degree of toxicity. Normal blood flow continues without indication of injury through capillaries stained a deep blue by long-continued perfusion. Its passage is not entirely unaccompanied by vital staining but this is relatively slight in an experiment requiring only a minimal passage through the capillary wall. The solution introduced consisted of 0.015 molar toluidine blue dissolved in standard

[1] The dyes used were vital stains prepared by the National Aniline and Chemical Company, New York, with the exception of trypan blue, which was a Grübler preparation.

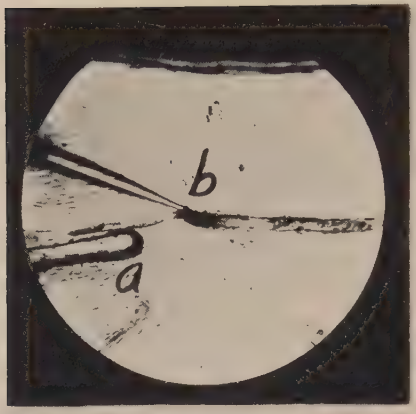

Fig. 1

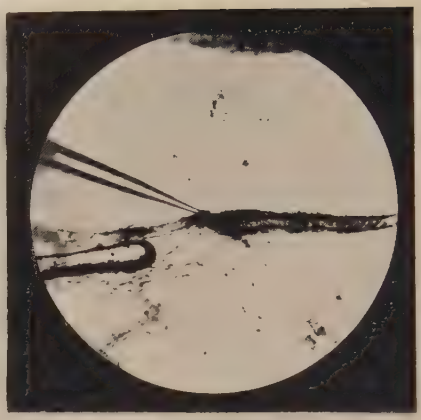

Fig. 2

Fig. 3

Fig. 4

Fig. 1. Photomicrograph (× 80) showing perfusion of a capillary with 0.005 M brilliant vital red in Ringer's fluid. *a* is the blunt glass rod by which circulation is stopped; *b* is the tip of the micro-pipette through which the dye is introduced and capillary pressure measured.

Fig. 2. The same capillary two minutes later, after the dye has passed through the wall. Capillary pressure was 18 to 19 cm. water.

Fig. 3. Photomicrograph (× 80) showing capillary after stoppage of flow at *a* with blunt rod. Within 2 minutes the corpuscles had moved inward as shown, indicating filtration of fluid. Capillary pressure was 26 cm. water.

Fig. 4. Photomicrograph (× 70) showing a second capillary similarly treated. Corpuscles had not changed their position at the end of 2 minutes. Capillary pressure was 9.5 cm. water.

frog Ringer's solution. Protocol 1 describes a typical experiment in which a series of capillaries were thus injected:

Protocol 1. June 6, 1926. Medium sized bull-frog pithed in brain only and prepared without loss of blood. Circulation in the mesentery very rapid and pressure generally high. Injected toluidine blue 0.015 molar in Ringer's solution.

Inj. 1. Mean pressure 30 cm. water, the capillary being directly connected to an arteriole
Dye appeared outside the capillary wall in one area after 5 seconds perfusion, and outside the entire vessel after 10 seconds
Diameter 19 micra
Pressure at end still 30 cm.

Inj. 2. Venous capillary pressure 16 cm.
Dye outside after 90 seconds
Diameter 32 micra
Pressure 16 cm.

Inj. 3. Pressure 12 cm.
Diameter 33 micra
Dye outside the capillary after 105 seconds
Pressure 14 cm.

Inj. 4. Pressure 18 cm.
Dye outside 22–26 seconds
Diameter 25 micra
Pressure 18 cm.

Inj. 5. Pressure 10 cm.
Dye outside in 150 seconds
Diameter 25 micra
Pressure 11 cm.

Inj. 6. Pressure 8 cm.
No visible passage after 300 seconds' perfusion
Diameter 30 micra
Pressure 7.5 cm.

Using this method 116 injections of individual capillaries were made in the mesenteries of 27 frogs. The observations have been arranged in groups according to *a*, the diameter of the capillaries injected, and *b*, the capillary pressure at which the perfusion was carried out.

In table 1 the results are arranged according to the diameter of the injected vessels. When perfusion had been continued for five minutes without passage of dye the experiment was ended and the time recorded as 5.0+ minutes. In the table there is shown the number of observations in each group, the variation in time of perfusion required, the average time, and finally the average pressure.

The times required ranged from 0.1 minute to 5.0+ minutes. As may be seen when the observations are arranged according to capillary diameter extremely high and low values are found in each of the groups. The figures have been plotted in figure 5 where it appears that the average time for the appearance of dye outside the vessel is fairly constant in capillaries of increasing diameter. There is no evidence therefore in these experiments of a measurable increase in permeability with dilatation of the capillary.

TABLE 1

DIAMETER OF CAPILLARY	NUMBER OF OBSERVATIONS	TIME REQUIRED FOR VISIBLE PASSAGE			AVERAGE CAPILLARY PRESSURE
		Lowest	Highest	Average	
micra		*minutes*	*minutes*	*minutes*	*centimeters*
11–14	11	0.1	5.0+	1.8	15
15–18	37	0.2	5.0+	2.3	14
19–22	11	0.2	5.0	2.4	12
23–25	28	0.4	5.0+	1.9	11
26–33	12	0.1	4.0	1.8	12
34–37	2	1.4	2.1	1.7	10

TABLE 2

CAPILLARY PRESSURE	NUMBER OF OBSERVATIONS	TIME REQUIRED FOR VISIBLE PASSAGE			AVERAGE DIAMETER
		Lowest	Highest	Average	
centimeters		*minutes*	*minutes*	*minutes*	*micra*
7– 8.5	14	4.1	5.0+	4.8	21
9–10.5	22	2.2	5.0+	3.1	24
11–12.5	26	1.0	4.0	2.2	23
13–14.5	21	0.5	3.5	2.1	20
15–16.5	14	0.5	3.0	1.5	19
17–18.5	4	0.3	1.5	0.8	23
19–20.5	3	0.3	0.7	0.5	17
21–22.5	4	0.1	0.5	0.3	16
23–24.5	4	0.2	0.7	0.5	18
25–26.5	2	0.1	0.5	0.3	17
27–30.5	2	0.1	0.2	0.15	18

An objection to this conclusion might be raised on the ground that the vessels perfused were merely capillaries of different size, not of varying degrees of dilatation. However, when the mesentery is first exposed to view practically all the vessels are constricted to such an extent that even single corpuscles have great difficulty in passing, but within ten minutes after exposure nearly all are dilated to widely varying degrees. During the hour or two following this first reaction to exposure the number of constricted vessels again increases considerably; and since it was during this

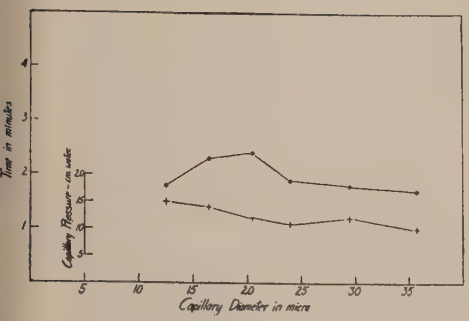

Fig. 5

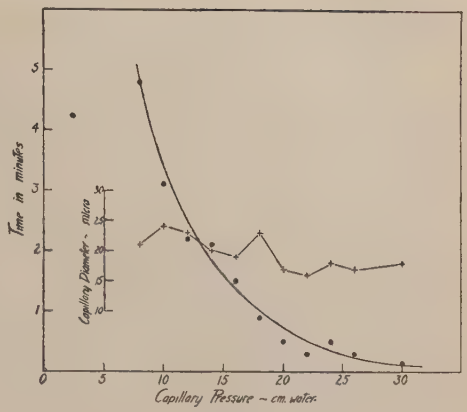

Fig. 6

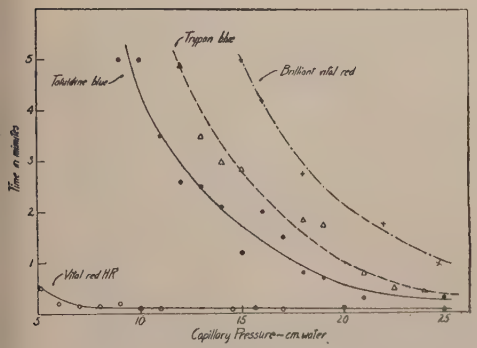

Fig. 7

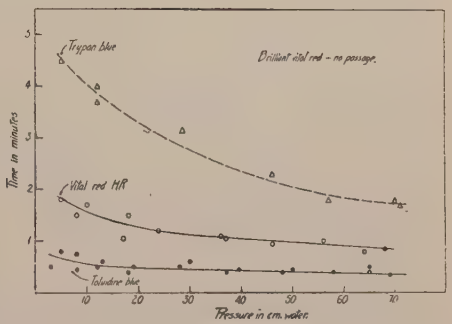

Fig. 8

Fig. 5. Chart showing the relation between capillary diameter and the rapidity with which 0.015 M toluidine blue in Ringer's fluid passes through the capillary wall.

Abscissae indicate capillary diameter in micra. Upper line designates average time of perfusion required for visible passage; lower line the average capillary pressure in each group of observations.

Fig. 6. Chart showing the relation between capillary pressure and the rapidity with which 0.015 M toluidine blue in Ringer's fluid passes through the capillary wall.

Abscissae indicate capillary pressure in centimeters water. Smooth curve shows the average time of perfusion required for visible passage; horizontal line indicates the average capillary diameter in each group of observations.

Fig. 7. Chart showing the relation between capillary pressure and the rapidity with which vital red HR, toluidine blue, trypan blue, and brilliant vital red, each in 0.005 M solution, pass through the capillary wall.

Abscissae indicate capillary pressure; ordinates show the time of perfusion required for visible passage of dye.

Fig. 8. Chart showing the permeability of collodion capillaries (8 per cent collodion in 80 per cent alcohol and 20 per cent ether) to toluidine blue, vital red HR, and to trypan blue. Brilliant vital red did not pass.

Abscissae indicate perfusion pressure; ordinates show the time required for visible passage.

period that nearly all the observations were made, the vessels injected were really in varying stages of dilatation and secondary constriction.

When, however, the results are grouped according to the capillary pressure at the time of perfusion the relation of dye passage to capillary pressure is clearly indicated, as is shown in table 2. It may be observed in figure 6 that at a pressure of 9 cm. or less in the majority of cases no dye passes through the wall even after 5 minutes' continuous perfusion. With rise in pressure there is a rapid decrease in the time required, so that at 15 cm. the average is 1.5 minutes, while at 30 cm. 10 to 15 seconds are sufficient. The average diameter of the capillaries remains fairly constant so that the correspondence with capillary pressure seems quite clear.

It appears from these results therefore that the rate of passage of toluidine blue in Ringer's solution is dependent upon the level of capillary pressure rather than upon the degree of dilatation.

Comparison of vital red HR, toluidine blue, trypan blue and brilliant vital red in their relation to capillary pressure. In order to determine whether the behavior of other substances than toluidine blue would show the same relationship to capillary pressure an identical procedure has been used with a series of vital dyes. Of those tested, some, such as rose bengale, could not be used because of the toxicity of the samples available. Others, such as methylene blue, neutral red and Congo red pass through the wall very unevenly due to their affinity for certain cellular elements outside the capillary.

The four dyes chosen for comparison were vital red HR, toluidine blue, trypan blue and brilliant vital red. Each was dissolved in Ringer's solution in the strength of 0.005 molar. The results of the capillary perfusions with these solutions are shown charted in figure 7. It seems clear that the four dyes are similarly affected by capillary pressure, in that passage is more rapid at the higher levels. They differ markedly, however, in the time required for the passage of a visible amount at any given capillary pressure.

Vital red HR (trypan red) was found by Schuleman (1917) to be one of the most diffusible members of the benzopurpurin group. When introduced into the capillary by micro-injection it passes through the wall almost immediately even at very low pressures. Its passage can be markedly delayed, however, if the colloid osmotic pressure of the dye solution is increased with gum arabic or gelatin. This effect can hardly be due to chemical combination with the colloid since diffusion into gelatin still occurs readily from these solutions, indicating the presence of dye in diffusible form. With 1 per cent gelatin solution the dye does not pass unless the pressure is in excess of 7 to 9 cm. water. As with toluidine blue there is no perceptible diminution in the permeability of the capillary wall with decrease of diameter.

Toluidine blue in the same concentration does not pass at pressures below 9 to 10 cm. Phenolsulphonephthalein and methylene blue apparently lie between vital red and toluidine blue. The former could not be used since change in reaction causes it to take on an orange tint as it passes through the wall, the latter because of its affinity for the Rouget cells.

Trypan blue in Ringer's solution requires a pressure in excess of 12 to 13 cm. for passage, filtering therefore with less ease than toluidine blue. According to other observers it passes only with moderate readiness (Edwards, 1926; Grollman, 1926) or with relative difficulty (Schulten, 1925) through the glomerular capillaries. Though passing more slowly than toluidine blue at low pressures, its rapidity of filtration is much increased by a rise of capillary pressure.

Brilliant vital red, used by Krogh and Harrop (1921) and by Krogh (1922) passes with the least facility, very little appearing outside the vessels at pressures below 14 to 15.5 cm. Even at 25 cm. a minute's perfusion is necessary to produce visible passage. Its rate of filtration, however, shows the same correlation with capillary pressure and the same lack of relation with capillary dilatation as was observed with each of the other dyes. A protocol of a typical experiment is given:

Protocol 2. December 7, 1926. Large female leopard frog, pithed in brain only. Ovaries and eggs removed after ligation of the pedicle. Circulation normal.

Inj. 1. Pressure first 16 cm., then 14 cm.
Diameter 19 micra
Passage in one portion of the vessel at 3.5 minutes, but in remainder no passage at 5 minutes

Inj. 2. Pressure 22.5 cm.
Diameter 25 micra
Passage in 2.5 minutes

Inj. 3. Pressure 15.5 cm.
Diameter 24 micra
Faint passage at 4.5 minutes, clear at 5 minutes

Inj. 4. Pressure 21 cm.
Diameter 11 micra
Passage at 2.75 minutes

Inj. 5. Pressure 14 cm.
Diameter 15 micra
None at 5 minutes

Inj. 6. Pressure 12.5 cm.
Diameter 18 to 25 micra
None at 5 minutes

Inj. 7. Pressure 12, 13, finally 13.5 cm.
Diameter 26 micra
No passage at 5 minutes

Inj. 8. Pressure 14 cm., then 16 cm.
Diameter 25 micra
Very faint passage at 4.5 minutes

Inj. 9. Pressure 18 cm.
Diameter 27 micra
Passage in 3.5 minutes

Physical properties of the dye solutions. The differences in the facility with which these four dye solutions pass through the capillary wall are apparently not related to their rate of diffusion into gelatin. Into 5, 20 and 30 per cent gelatin, toluidine blue diffuses far more rapidly than vital red HR. Trypan blue diffuses at a very slightly slower rate than vital red HR, while brilliant vital red is almost entirely indiffusible. Nor can the differences be related to the acid or basic nature of the dyes, since two acid dyes, vital red HR and phenolsulphonephthalein, pass more rapidly; while two others, trypan blue and brilliant vital red, pass more slowly, than do the basic dyes, methylene blue and toluidine blue.

According to Michaelis (1905) semi-colloidal and colloidal dyes probably exist in two states, one dissolved and the other colloidal, the proportions varying with concentration. From this property it might be expected that those dyes which apparently prevent the filtration of water below a certain pressure would show evidences of colloidal behavior. This has been found to be the case insofar as brilliant vital red, which passes only at pressures above 14 to 15 cm., produces by far the most perfect Tyndall cone when a beam of light is transmitted by the dye solution. Trypan blue, though too opaque in 0.005 molar solution to permit observation, produces a somewhat less clear cone in more dilute solution. With toluidine blue there is noted a clear beam of reddish light—the fluorescence which is described by Michaelis. In the case of vital red, however, though examined in 0.005 M concentration, no Tyndall cone was visible. This, coupled with the fact that it is the only dye which was found to pass freely at low pressures, suggests that its lack of colloidal properties may possibly explain its behaviour compared to other dyes.

Moreover, if vital red HR and trypan blue each in 0.005 M strength in Ringer's fluid are introduced together into a capillary, no dye whatever passes at pressures below 12 cm. Above this level a violet coloration results. Yet diffusion in gelatin separates out to some extent the red from the blue dye. It seems probable that water and the substances in true solution, including the vital red HR, are retained in the capillary lumen by the osmotic pressure of the colloidal portion of the trypan blue at pressures below 12 cm. water.

Comparison of capillaries and collodion tubes in relation to dye passage. To test further the osmotic properties of the dye solutions, capillaries of collodion were prepared. Threads of sugar were formed by "pulling" a plastic mass of heated dextrose. The threads were cooled and then immersed in collodion of the three grades of permeability used by Krogh (1922). After drying in the air for 3 minutes the thread of sugar, coated with collodion, was placed in 60 per cent alcohol, which dissolved out the dextrose, leaving a collodion capillary between 100 and 300 micra in diameter and about 10 cm. in length with two flaring ends measuring 3 to 4 mm. in diameter. A glass tube was tied into each end and the capillary was mounted in a holder to prevent undue tension.

The outside of the capillary was bathed in Ringer's solution while the dye solutions described above were perfused through the lumen. One area of the collodion tube was observed under the microscope as the dye solution flowed and the time for barely visible passage was recorded as for the mesenteric capillaries. Flow was maintained constant while pressure was varied between 5 and 80 cm. of water. Toluidine blue passes with greater rapidity and at lower pressures than any of the acid dyes. Vital red HR is the only acid dye of the three studied which passes through the wall of a capillary made of 8 per cent collodion dissolved in equal parts of ether and alcohol.

Capillaries made of 8 per cent collodion in 80 per cent alcohol and 20 per cent ether are far more variable in their degree of permeability and more difficult to duplicate. They permit practically immediate passage of toluidine blue, a somewhat slower passage of vital red, and a still more delayed passage of trypan blue (fig. 8). They are entirely impermeable to brilliant vital red.

It is suggestive that the acid dyes show the same order in their facility of passage as was observed with the capillary wall of the frog. The negative charge on the collodion membrane is possibly the complicating factor which delays the passage of vital red HR, trypan blue, brilliant vital red and apparently all acid dyes. Thus the wall of the collodion capillary permits the basic dye, toluidine blue, to pass far more rapidly than vital red HR. This difference between the collodion capillary and the endothelial wall of mesenteric capillaries confirms the findings of Grollman (1926) who observed that the comparison of the capillary wall with a simple collodion membrane as a filter for dyes was not justified.

It seems probable from the physical properties described above that the differences observed in the four dye solutions are to be explained on the basis of their colloidal properties and their osmotic activity with reference to the endothelium as a filtering membrane.

The passage of vital red HR injected into the blood stream. Vital red HR was the only dye to which the capillary endothelium appeared to be wholly

permeable. It can be introduced into the blood stream to a visible concentration apparently without increasing the colloid osmotic pressure of the plasma. It was therefore injected as a saturated solution into the blood passing through the ventricle until the plasma in the mesenteric vessels had a definitely pink color. One capillary was selected for observation of the passage of the dye. At the end of five minutes' observation a micro-pipette was inserted into a collateral vessel and capillary pressure measured while flow still continued.

It was found that in those capillaries where no dye passed the pressure was always below 11 cm. Where passage was rapid the pressure was almost always 14 cm. or over. In any case, diameter played a part only indirectly, since dilated capillaries with high pressure seemed quite permeable, while other dilated vessels with low pressure seemed impermeable.

In animals which had suffered severe hemorrhage and in which capillary pressure was very low there was no passage of dye. It moved only slowly or not at all through the walls of constricted vessels, where pressure is usually low. If, however, the total circulation of an arteriole was diverted, by means of the microscopic glass rod, into such a vessel thus increasing its pressure, the dye passed very quickly. Frequently during the first five minutes it did not come out equally along the entire length of any one capillary but in greater amount outside the arterial portion of the vessel.

Injury of the wall leads to a rapid filtration of the dye, often with the production of stasis, which is easily explicable on the basis of a lowered effective colloid osmotic pressure, due to the increased permeability of the injured endothelium. Application of urethane similarly causes passage at lower pressures owing to injury of the wall (Landis, 1927b). These results can best be explained on the view that there is in the plasma a certain amount of freely diffusible dye which acts as an indicator of the filtration of fluid. At capillary pressures below the colloid osmotic pressure of the plasma the flow of fluid is inward, but above this level fluid and the dissolved dye pass outward and cause staining.

This relationship holds, however, only during the first ten minutes following the introduction of the dye. After this time the results are obscured by the rapid vital staining of the connective tissue around the smaller venous capillaries and particularly the smallest venules. These are stained deeply irrespective of pressure or diameter. Nevertheless the behaviour of the dye during the first five minutes before vital staining occurs provides further qualitative evidence in favor of the importance of capillary pressure in fluid movement.

II. The direct determination of the rate of fluid movement through the capillary wall in relation to capillary pressure. In earlier experiments it was noted that after injury of the capillary a concentration of cells, indicating filtration of fluid through the wall, occurs

slowly or not at all when capillary pressure is low, and rapidly when the pressure is high. These observations have been extended to normal capillaries in such a way as to permit the measurement of the absolute rate at which fluid moves through a unit area of capillary wall at various pressures.

If the flow of a rapidly moving stream of blood is stopped by gently compressing the capillary with the blunt glass rod, in some instances the corpuscles rapidly move inward toward the closed end (fig. 3), in others they move outward, and in still others (fig. 4) no change can be observed. The forces involved are *a*, the hydrostatic pressure exerted by the blood stream at the open end of the capillary, tending to cause filtration; and *b*, the osmotic pressure of the plasma proteins in the imprisoned blood which tends to prevent filtration or to bring about absorption of fluid. According to the Starling hypothesis filtration should occur in those capillaries where hydrostatic pressure is high, absorption where it is low, and neither when the two pressures are approximately balanced. This appears to be the case.

With the capillary closed at one extremity, as long as the diameter remains constant and the corpuscles move freely with the plasma, filtration of fluid causes the corpuscles at the open extremity to move inward toward the closed end. With absorption of fluid the corpuscles at the open end of the capillary are displaced outward to make room for the added volume of fluid within the vessel.

To measure the rate of movement of the corpuscles at the free end of such a closed capillary an ocular micrometer scale, 660 micra in length and divided into 20 units, is placed parallel to the capillary. The lumen of the latter is closed by gentle pressure with the rod opposite one end of the scale (*a*, fig. 3) and the eye at the same time is fixed upon one edge of a corpuscle at the other end of the scale (*b*, fig. 3). The movement of this corpuscle is followed for a period of 60 to 100 seconds and the times at which it passes each mark on the scale are recorded by signal magnet and kymograph. At the end of the period of observation a micro-pipette is introduced into the capillary and the pressure is measured with the blunt rod still in position. Record is made of capillary pressure, the diameter of the vessel, the position of the corpuscle at the outset, and the times required for it to move past the markings of the ocular micrometer (see protocol 3 below).

Figure 9 shows the relation between the rapidity and direction of the movement of the corpuscle at the open extremity in relation to capillary pressure, which is given at the right of each curve. The experiments involve capillaries of approximately the same length and diameter in order to roughly eliminate differences in filtering area and volume change. Filtration is indicated by the inward movement of the corpuscles, which, as the chart shows, occurs at an increasingly rapid rate as capillary pres-

sure rises above 11.5 to 12.5 cm. Absorption of fluid, indicated by the outward movement of the corpuscles, becomes more rapid as pressure falls below 9 to 9.5 cm.

In each experiment the movement of the observed corpuscle is at first rapid, then becomes slower, and finally at a certain middle range of pressures ceases altogether. This apparently indicates a gradual concentration or dilution of the plasma colloids until their new osmotic pressure corresponds to the capillary pressure. Even this roughly qualitative treatment of the data makes it appear that the osmotic pressure of the plasma proteins must lie somewhere between 9 and 12.5 cm. water, and that as capillary pressure rises above this point there is filtration of fluid, and as it falls below it, absorption. This relationship can be indicated more exactly by computing the unit rate of fluid movement at various capillary pressures.

Computation of the rate at which fluid passes through the capillary wall. With the first change in the volume of the blood imprisoned in the capillary the osmotic pressure of the plasma colloids is no longer at the level normal for freely flowing blood. It is therefore the volume change at the first instant which must be used to compute the rate of filtration from unchanged blood. This is obtained by drawing for each experiment a curve of the type shown in figure 9. A tangent to this curve at zero time will give the rate at which the corpuscle was moving while the osmotic pressure of the plasma proteins was still essentially normal. This figure (micra per second) multiplied by the cross-sectional area of the capillary will indicate the rate (in cubic micra per second) at which fluid was being filtered or absorbed through the observed length of capillary by normal blood.

The area of filtering surface at zero time is computed by finding the area of the cylinder of endothelium extending from the closed end of the capillary (*a*, fig. 3) to the position of the observed corpuscle at the beginning of the experiment (*b*, fig. 3). The volume change per second divided by the filtering area (in square micra) will give the unit rate of fluid movement in cubic micra per square micron of capillary wall per second. In protocol 3 is shown in tabular form the observed values and computations of a typical experiment.

Seventy capillaries in the mesenteries of 11 frogs have provided the results shown in figure 10, in which the unit rate of fluid movement is plotted against capillary pressure, filtration being designated + and absorption −. The disproportionately large number of points on the zero line is due to the fact that in those experiments in which the corpuscles moved less than one micrometer unit (33 micra) it was necessary to record the rate of movement as zero.

The sources of error lie chiefly in the lack of absolute uniformity of

capillary diameter through the entire length of the vessel observed, and in the possibility that during the 30 to 60 seconds elapsing between the measurement of fluid movement and the determination of pressure there may be some change in the latter, though this difficulty was guarded against by observing the rate of flow in collateral vessels, which has been shown to follow faithfully the fluctuations of pressure over short periods of time.

Protocol 3. February 15, 1927. Small-sized female leopard frog. Ovaries and eggs removed after ligation of pedicle. Mesentery prepared without blood loss. Circulation moderately rapid.

CAPILLARY NUMBER	CAPILLARY PRESSURE	DIAMETER	POSITION OF CORPUSCLE	TIME	RATE OF MOVEMENT	RATE OF VOLUME CHANGE	FILTERING AREA	UNIT RATE OF FLUID
	cm. water	*μ*	*μ*	*seconds*	*μ/second*	*cubic μ/second*	*square μ*	*cubic μ/square μ/second*
1	26	18	660	0	9.4	2,390	37,300	0.064
			627	3.5				
			594	7.0				
			561	10.5				
			528	14.0				
			495	18.0				
			462	22.0				
2	20.5	27	594	0	4.7	2,690	50,400	0.053
			561	8.5				
			528	30.0				
3	15.7	22	660	0	2.2	840	45,600	0.018
			627	12.5				
			594	31.0				
			561	43.0				
4	13.3	17	495	0	0.5	110	26,440	0.004
			479	52.0				
			Remained stationary					
5	17.0	25	660	0	3.8	1,880	51,840	0.036
			627	8.0				
			594	19.0				
			561	27.0				
			528	39.0				
6	8.0	15	495	0	−1.4	−243	23,300	−0.011
			511	13.0				
			528	32.0				
7	13.7	20	495	No movement		0	31,100	0

In addition the spread of observed values is in part probably due to the fact that the blood which is imprisoned within the capillary varies according to its source and previous rate of flow. The greater volume changes are observed when the rate of flow, preceding the experiment, is rapid, or when the blood enters the capillary directly from an arteriole; since in either case there is less opportunity for previous fluid interchange. Slow flow, particularly through a long capillary, permits the blood to come partially into equilibrium with the local capillary pressure and therefore diminishes the rate of filtration observed when flow is stopped in the experiment. For this reason it seemed necessary to make all determinations in vessels in which flow was at least moderately rapid.

As indicated in figure 10 at pressures between 29 and 14.5 cm. the movement of fluid is outward in all cases. Between 14 and 12.5 cm. fluid passage occurs very slowly in an outward direction or is absent, the latter state indicating a condition of equilibrium. From 12 to 7 cm. movement is absent or inward, and below 7 cm. inward movement is observed in all cases.

At 25 cm. pressure the observed rates of filtration range from 0.045 to 0.068 cubic micra per square micron of capillary wall per second, while at 15 cm. they vary from 0.012 to 0.018. The large majority of observations between pressures of 9 and 13 cm. show no movement of fluid in either direction. At 5 cm. pressure fluid is absorbed at rates between 0.020 and 0.050 cubic micra per square micron per second.

As would be expected from Poiseuille's law, the observed points are dis-

Fig. 9. Chart showing the manner in which capillary pressure determines the rate and direction of movement of the corpuscles at the open extremity of the occluded capillary. Vessels of similar length and diameter have been chosen to make volume changes approximately the same.

Ordinates indicate movement of corpuscles in arbitrary units, each equivalent to 33 micra. With filtration of fluid the cells move inward toward the closed end; with absorption the increase in plasma volume causes the cells to move outward toward the open extremity. Abscissae show time in seconds.

Fig. 10. Chart showing the rate of fluid movement in relation to capillary pressure.

Ordinates indicate rate of fluid movement in cubic micra per square micron of capillary surface per second. Positive values denote filtration of fluid, negative values absorption. Abscissae show capillary pressure in cm. water. The equation for the line is $x = 11.5 + 178\,y$.

Fig. 11. Chart showing the marked increase in rate of passage of brilliant vital red through the walls of capillaries injured by ether to the point of stasis.

Abscissae denote capillary pressure in cm. water. Ordinates indicate the time of perfusion required for visible passage of dye.

Fig. 12. Chart comparing the rates of filtration of fluid through normal endothelium (below) and the walls of capillaries killed by 10 per cent alcohol (dots) and mercuric chloride 1:10,000 (crosses).

Ordinates indicate rate of fluid movement in cubic micra per square micron capillary wall per second. Abscissae indicate capillary pressure in centimeters of water

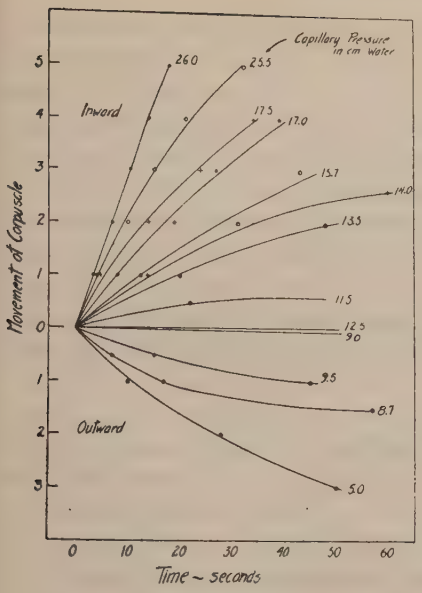

Fig. 9

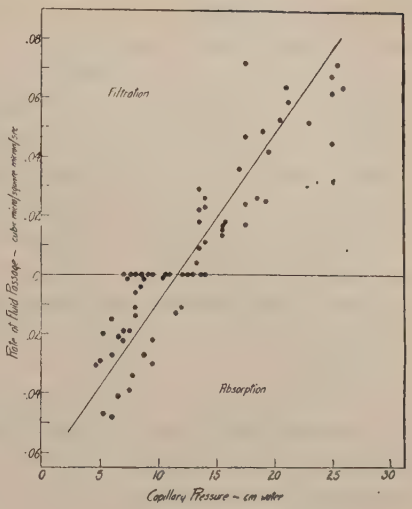

Fig. 10

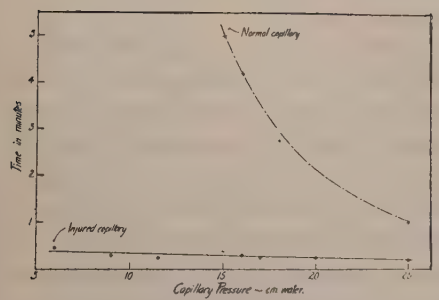

Fig. 11

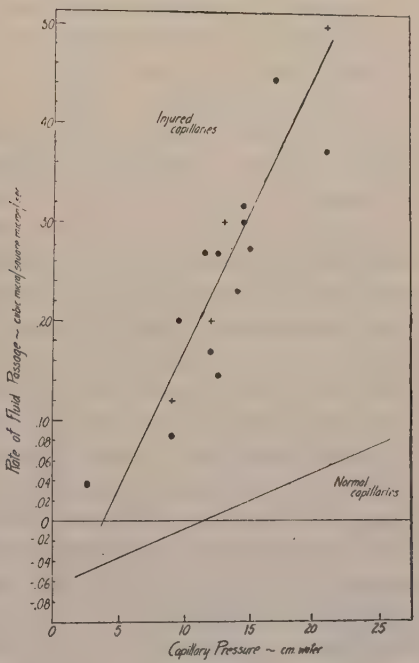

Fig. 12

tributed about a straight line. This line, computed by the method of least squares, has an equation $x = 11.5 + 178\ y$, where x is capillary pressure and y is the rate of filtration and therefore intersects the line of zero filtration at 11.5 cm. pressure. This represents the capillary pressure in cubic centimeters of water at which fluid moves neither inward nor outward. Since by definition this is osmotic pressure, it is believed that it represents the osmotic pressure of the plasma proteins against their normal filtering membrane. The slope of the line indicates the rate of movement of fluid in relation to the difference between the colloid osmotic pressure and capillary pressure, amounting to approximately 0.03 cubic micra per square micron capillary surface per second for a difference of 5 cm. water pressure.

This method can therefore be used under various experimental conditions to indicate the permeability of the capillary wall with reference to a, the rate of movement of fluid, and b, the effective osmotic pressure of the plasma proteins.

III. The permeability of the injured capillary wall determined by experiments of both types. There is evidence that following injury of the capillary wall by certain means permeability is increased to such an extent that the plasma colloids can pass with ease (Jacobj, 1921· Osato, 1922; Tainter and Hanzlik, 1924). The higher concentrations of urethane, alcohol, ether, etc. (Landis, 1927b) appear to injure the endothelium to such an extent that the plasma with its proteins is almost entirely drained from the blood in the capillary vessels, leaving solid masses of closely packed cells—the condition to which Krogh (1922) has applied the term of "stasis."

With dyes the effect of various toxic substances on the capillary wall is easily demonstrated to be in the direction of greater permeability. The toluidine blue solution above described passes through the injured wall in 5 to 6 seconds at all pressures, even those below 9 cm., at which level the normal capillary, due possibly to the semi-colloidal nature of the dye, permits no visible passage. The same can be observed with trypan blue and brilliant vital red. For the latter, figure 11 shows the time of perfusion required at various pressures to produce visible passage of dye through the walls of normal capillaries and of those injured to the point of stasis by Ringer's solution saturated with ether. The same results are obtained when the dye solution is saturated with ether and injected into normal capillaries. With injury the endothelium seems to become quite permeable to semi-colloidal dyes, as well as to the plasma colloids, at all pressures.

The method of direct measurement of fluid passage has also been applied to injured capillaries. The mesentery was exposed to a constant flow of 10 per cent alcohol in Ringer's fluid. After this had continued for 10

minutes the flow of blood through the capillary network usually became more sluggish, and the corpuscles appeared to be more concentrated, indicating an increase of permeability. When flow was then stopped, in the manner described above, in a series of single capillaries, a very rapid concentration of cells was noted, even at pressures as low as 4 to 5 cm. of water. The unit rate of filtration as determined for a moderate range of capillary pressure is indicated in figure 12. As with the normal vessels the points are distributed about a straight line, but the line is shifted to the left and the slope increased. The equation is $x = 4.0 + 26\ y$, indicating that filtration occurs at approximately seven times the rate determined for normal capillaries, for a given difference between the effective osmotic pressure of the plasma proteins and capillary pressure.

In the normal capillary the concentration of cells never proceeds to the point where practically all of the plasma is filtered off, due to the mounting colloid osmotic pressure, which finally comes into equilibrium with the capillary pressure. In the injured vessels, however, the cells become concentrated into a solid cylinder, indicating the loss of practically all the fluid in the capillary and therefore of the plasma proteins.

That the permeability to colloids is increased is indicated by the fact that the straight line no longer crosses the line of zero filtration at 11.5 cm. as in the normal vessel, but at some pressure well below 5 cm. With considerably more difficulty the mesenteric vessels have been killed with 1:10,000 mercuric chloride solution. When, after this treatment, the rates of filtration are observed just as stasis is appearing, they fall upon the same line as those for 10 per cent alcohol, indicating that killing with these two substances produces essentially the same degree of increase in permeability.

IV. Discussion. The average pressure in the arteriolar capillaries of the frog's mesentery is 14.5 cm. and in the venous capillaries 10.0 cm. water. The direct measurement of fluid movement indicates that at pressures above 11.5 cm. the passage is outward and below that point inward. This relationship supports the theory of Starling that in general the direction of fluid movement is outward toward the tissues in the arteriolar end of the capillary network, and inward toward the blood in the venous portion.

White (1924) using collodion membranes as osmometers has placed the osmotic pressure of the plasma proteins for frog's blood between 10 and 12 cm. of water. The experiments here described, in a sense, make of each capillary an osmometer in which the osmotic pressure of the imprisoned blood appears to have an average value of 11.5 cm. It seems that for the mesenteric capillaries qualitative experiments with dyes, quantitative determinations of fluid movement, and the determinations of osmotic pressure all indicate the existence of a balance between capillary pressure and the osmotic pressure of the plasma proteins.

Another view of capillary permeability, in many ways opposed to that just discussed, must be considered. Krogh and Harrop (1921) have found that with the dilatation produced by the narcotic urethane in concentrated solution the permeability of the capillary wall increases to such a degree that plasma with its colloids drains off completely, leaving the corpuscles tightly packed together within the vessel. Capillary pressure was not measured but was inferred to undergo no change. Krogh (1922) concludes that: "No dilatation of capillaries involving mechanical stretching of the endothelium can take place without being accompanied by an increase in permeability, an increase which runs, on the whole, parallel to the degree of dilatation and which allows all the normal plasma colloids to filter off rapidly when the capillaries are strongly dilated." Other experiments led to the further generalization that "there is in almost all tissues an excess of the effective osmotic pressure over capillary blood pressure."

In the first group of experiments, however, it has been shown that there is no evidence of an increase in permeability with increasing diameter. On the contrary, capillary pressure measurements explain satisfactorily the variation in the rate of passage in different vessels. A study of the stasis produced by urethane (Landis, 1927b) has made it appear that the effect of this drug is due to injury, and is comparable to the increase of permeability obtained above by killing the endothelium, where also the wall became permeable to colloids. Finally there seems to be some indication that in the vessels studied there is a balance between average capillary pressure and colloid osmotic pressure. The evidence at hand indicates that dilatation does not increase the permeability of the capillary wall directly, though it may appear to do so in active hyperemia where there is a concomitant rise of capillary pressure.

Hirschfelder (1924) found also that in the edema produced by mustard oil vaso-dilatation increases the edema only when it also raises capillary pressure. Thus sodium nitrite locally increases the inflammatory effect of the irritant, but intravenous injection so lowers systemic blood pressure that the capillary pressure is insufficient to produce edema. Epinephrin inhibits the development of edema on the treated side for about two hours, at the end of which time the vaso-constriction due to the drug disappears. The lessened edema due to both this drug and to cocain are ascribed to a diminution of filtration pressure.

The importance of capillary pressure is noted also by Tainter and Hanzlik (1924) in their study of paraphenylenediamine edema. Loss of fluid could be prevented by a marked fall in systemic blood pressure and also by the administration of epinephrin. The latter raises systemic blood pressure but both procedures have the same effect on capillary pressure. Injection of pituitrin, which resembles epinephrin in its effect upon blood pressure, has been shown by Bayley, Davis, Whitman and Scott

(1925) to diminish the volume of lymph collected from the thoracic duct, and to delay the exit of physiologic salt solution from the blood stream. These observations emphasize the fact that it is not sufficient in studies of capillary permeability to record blood pressure and—as is often done—to assume that its changes indicate fluctuations of capillary pressure, since the two may vary independently and often in opposite directions.

Studies of capillary permeability are made difficult by the constantly varying diameter, pressure and flow in the vessels which form the capillary network. The number of variables can be reduced and the separate factors better controlled when single capillaries are studied. The experiments here described, carried out on this principle, are believed to indicate in a quantitative manner the effect of changes in capillary pressure, as distinct from systemic pressure, upon the passage of fluid through the walls of the mesenteric capillaries in the frog. The relative magnitude of the effects observed, even in injured vessels, indicates that before attributing changes in the rate of fluid movement to a modification of the capillary wall itself, it is essential definitely to eliminate change in capillary pressure as a possible factor.

SUMMARY

1. Micro-injection of dye solutions into single capillaries in the frog's mesentery indicates that the walls of dilated capillaries are not more permeable than those of constricted vessels.

2. The rate of passage of a dye solution through the capillary wall appears to depend upon the level of capillary pressure, not upon capillary diameter.

3. At any given pressure various dye solutions differ markedly in the time required for visible passage, due probably to their different colloidal properties. So far as permeability to dyes is concerned comparison of capillaries made of collodion to the mesenteric capillaries is not justified.

4. Vital red HR introduced into the ventricular blood passes immediately through the walls of those capillaries whose pressure is above 14 cm. water, but is retained for some minutes at pressures below 11 cm.

5. By direct measurement the rate of fluid movement through the capillary wall was found to be directly proportional to the difference between capillary pressure and the osmotic pressure of the plasma proteins, amounting to approximately 0.03 cubic micra per square micron of capillary wall per second for a difference of 5 cm. water pressure.

6. The osmotic pressure of the plasma proteins against their natural membrane as indicated by this method is 11.5 cm. water. When capillary pressure exceeds this value, filtration occurs, when it falls below it absorption results.

7. Capillaries injured by alcohol and mercuric chloride appear to be

permeable to the plasma colloids and approximately 7 times more permeable to fluid than the normal capillary wall.

BIBLIOGRAPHY

Anitschkov, N. 1924. Klin. Wochenschr., iii, 1729.

Bayley, Davis, Whitman and Scott. 1925. Proc. Soc. Exper. Biol. and Med., xxii, 312.

Edwards, J. G. 1926. This Journal, lxxv, 330.

Grollman, A. 1926. This Journal, lxxv, 287.

Hirschfelder, A. D. 1924. This Journal, lxx, 507.

Jacobj, W. 1923. Arch. exper. Path. u. Pharm., xcviii, 55.

Krogh, A. 1922. Anatomy and physiology of the capillaries. Yale University Press.

Krogh and Harrop. 1921. Proc. Physiol. Soc., Journ. Physiol., liv, cxxv.

Landis, E. M. 1926a. This Journal, lxxv, 548.

1926b. Amer. Journ. Med. Sci., clxxi, 662.

1927a. Amer. Journ. Med. Sci., clxxiii, 449.

1927b. This Journal, lxxxi, 124.

Michaelis, L. 1905. Virchow's Arch. f. path. Anat. u. Physiol., clxxix, 195

Okuneff, N. 1924. Pflüger's Arch., cciv, 261.

Osato, S. 1922. Tohoku Journ. Exper. Med., ii, 465, 489.

Pohle, E. 1924. Pflüger's Arch., cciii, 558.

Rogowicz, N. 1885. Pflüger's Arch., xxxvi, 252.

Schuleman, W. 1917. Biochem. Zeitschr., lxxx, 1.

Schulten, H. 1925. Pflüger's Arch., ccviii, 1.

Schimidzu, Y. 1922. This Journal, lxii, 202.

Starling, E. H. 1896. Journ. Physiol., xix, 312.

Tainter and Hanzlik. 1924. Journ. Pharm. Exper. Therap., xxiv, 179.

White, H. L. 1924. This Journal, lxviii, 523.

Wittgenstein and Krebs. 1926. Pflüger's Arch., ccxii, 268.